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## Parathyroid Hormone coupled to Cell Penetrating Peptides for Oral delivery

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**Objective.** Oral delivery of peptide drugs constitutes a number of challenges including poor membrane permeability. To improve this, the use of cell penetrating peptides (CPPs) is of great interest as they have shown potential in improving the transepithelial transport of therapeutic peptides upon co-administration or direct conjugation. Knowledge is however lacking regarding the effect on the cell penetrating propensity of the CPP, for which an  $\alpha$ -helical content is important, as a result of direct conjugation.

Hence, the objective is to produce fusion-peptides comprising the biologically active part of parathyroid hormone (PTH(1-34)) coupled to different CPPs and characterize these according to secondary structure and ability to permeate an intestinal epithelium *in vitro*.

**Methods.** PTH(1-34)-CPP fusion-peptides were expressed in *E. coli* as inclusion bodies, solubilized and purified by affinity chromatography and RP-HPLC. The secondary structure was studied using circular dichroism (CD) spectroscopy and the delivery propensity was assessed employing the intestinal Caco-2 cell line.

**Results.** PTH(1-34) and different N- or C-terminally CPP-coupled PTH(1-34) were successfully produced. CD spectra revealed a disordered secondary structure in buffer, but in the presence of liposomes the  $\alpha$ -helical content increased in some but not all fusion-peptides. The specific N- or C-terminal positioning of the CPP was shown to be important for the transepithelial permeability, with N-terminal CPP-coupling resulting in significantly improved transport when compared to C-terminally CPP-coupled PTH(1-34). However, only one of the fusion-peptides was able increase the permeability over Caco-2 cell monolayers when compared to PTH(1-34) administered alone or co-administered with the CPPs.

**Conclusions.** Direct coupling of a CPP to a therapeutic peptide was investigated with respect to overall secondary structure and permeation across an intestinal epithelium, showing that both the  $\alpha$ -helical content and the transepithelial permeability were dependent on the specific CPP sequence and the specific N- or C-terminal CPP coupling.